The hyperdynamics of Lamin B observed in undifferentiated ESCs (Bhattacharya et al., 2009) is not represented in DamID. This finding may explain the relatively small differences observed between the various differentiation stages. It may also explain the lower overall dynamic range observed in ESCs compared to the other cell types, perhaps reflecting innate heterogeneity or, as the authors suggest, less robust NL interactions in ESCs. Nevertheless, many genomic regions significantly alter their nuclear positioning, concomitant with the expression level of the harbored genes. Therefore, even if genome-lamina interactions are more dynamic than can be captured by DamID, the technique still elegantly demonstrates functional reorganization of many parts of the genome during ESC differentiation.

Similar to the case of chromatin plasticity in ESCs and its causal relationship with transcriptional promiscuity (Mattout and Meshorer, 2010), here too the authors argue causality to be an open question and entertain at least two mechanistic possibilities. Intuitively, proximity and subsequent association of LADs with the NL could result in spatial regulation of lineage specific gene expression; nevertheless, it is quite plausible that when lineage-specific transcriptional programs activate or repress a certain locus, this locus in turn recruits (or is recruited to) the NL as a spatial coregulator of expression. This speculation, however, remains to be demonstrated.

The role of lamin A in lamina-related silencing is an intriguing open question. In somatic cells, when genomic loci are silenced by their tethering to the nuclear lamina, lamin A accumulates at the tethered site (Reddy et al., 2008), possibly participating in the silencing process. Therefore, it would be interesting to test this hypothesis in ESCs, where lamin A expression is absent and where the nuclear lamina seems to be more amorphous than in differentiated cells (Mattout and Meshorer, 2010). Along these lines, DamID in the presence and absence of lamin A can yield important insights on lamin A-related regulation at a genome-wide scale. It might also be worthwhile to develop tools, which will allow controlled expression of Lamin B-Dam at short intervals. Comparing several different short expression pulses of Lamin B-Dam may provide an additional dynamic dimension. Such DamID-related experiments together with genome-wide chromosome conformation capture (Hi-C) techniques (Lieberman-Aiden et al., 2009) should provide a global three-dimensional view of nuclear architecture and its association with the nuclear lamina in the imminent future.

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Multiple, Interconvertible States of Human Pluripotent Stem Cells

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Three recent studies, including Buecker et al. (2010), in this issue of *Cell Stem Cell*, report that human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can exist in distinct but interconvertible states and describe a robust expansion of human ESCs/iPSCs that resemble mouse ESCs.

Although human and mouse embryonic stem cells (ESCs) are derived from similar developmental stages with comparable methodologies, the resulting human and mouse ESC lines show overt differences in colony morphology, proliferation rate, growth factor requirements, and cell-surface marker expression. The stark differences between human and mouse

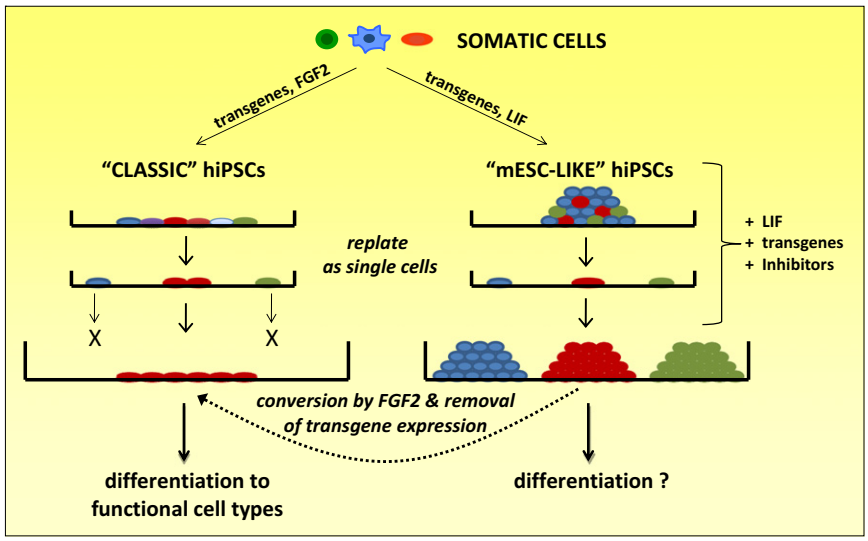

Figure 1. Potential Applications of Mouse ESC-like hiPSCs
Human somatic cells can be reprogrammed to either classic hiPSCs with five reprogramming transgenes and FGF2 (left) or to a mouse ESC-like state when FGF2 is replaced by LIF (right), as shown by Buecker et al. (2010). The compact, dome-shaped (mESC-like) colonies that form with LIF express different cell-surface markers and require LIF and transgene expression for propagation. Like mESCs, they demonstrate much higher clonal growth efficiency when plated as single cells, as well as higher proliferation rates. These mESC-like attributes facilitate transgenesis and gene targeting in human pluripotent cells, which is difficult in classic hiPSCs and hESCs.

ESCs are recapitulated in human and mouse induced pluripotent stem cells (iPSCs) reprogrammed from somatic cells. Recent publications, including one from Geijsen and colleagues in this issue of *Cell Stem Cell* (Buecker et al., 2010), now show that different culture conditions can be used to derive hiPSCs with mESC-like traits and to convert these rapidly growing cells to more conventional hiPSC lines.

The reason that human and mouse ESCs (and iPSCs) derived from a seemingly equivalent developmental stage behave so differently in culture presents a puzzle to stem cell biologists. These differences became even more intriguing after the generation of mouse and rat epiblast-derived stem cells or EpiSCs (Brons, et al., 2007; Tesar et al., 2007). Mouse and rat EpiSCs resemble hESCs in terms of morphology, growth factor requirement, and gene expression patterns. However, they are unable to form teratomas, a recognized assay for ESC-like pluripotency. Although the observed similarities between mouse EpiSCs and hESCs in culture are highly reproducible, their relevance to in vivo biology is debatable. One possibility is that EpiSCs (and thus hESCs) are less primitive or more primed than mESCs because EpiSCs are derived from a developmentally later stage. However, this linear (and mouse-centric) hypothesis, which tries to link in vitro cell states to sequentially in vivo developmental states of different species, cannot explain a number of well-established observations. For example, hESCs and mouse/rat EpiSCs, but not mESCs, can readily form cells that resemble trophectoderm, which, during development, diverges at the earliest blastocyst stage, prior to epiblast formation. An alternative hypothesis to explain the differences is that a pluripotent mammalian cell can exist in a limited number of phenotypically and genetically distinct states that are interconvertible in response to altered culture conditions. This "multiple in vitro state" hypothesis is supported by several recent studies that elegantly demonstrated that mouse ESCs, EpiSCs, and EGCs are interconvertible through changing culture conditions (Chou et al., 2008; Hayashi and Surani, 2009). Now a new wave of reports, including the findings from Geijsen and coauthors, show that human pluripotent cells can also exist in discrete and interconvertible states in culture (Buecker et al., 2010; Hanna, et al., 2010; Xu, et al., 2010).

Buecker et al. (2010) found that inducible expression of reprogramming transgenes can lead to the derivation of either colonies of standard human iPSCs (hiPSCs) when FGF2 was included or, alternatively, colonies with mESC-like properties when FGF2 was replaced by LIF (Figure 1). The mESC-like human cells, which they term hLR5 cells, proliferate more rapidly and with a higher clonal replating efficiency than standard hiPSCs, form compact dome-shaped colonies, and express the surface marker SSEA1 rather than the SSEA3/4 and TRA-1-60/TRA-1-81 antigens associated with classic hiPSCs and hESCs (Buecker et al., 2010). The mESC-like state of these reprogrammed human cells is substable, or "metastable," because their maintenance requires the continued expression of the ectopic reprogramming transgenes in addition to LIF/JAK/STAT3 signaling. The robust growth of mESC-like human cells facilitates efficient genetic manipulations such as transgenesis and homologous recombination (Buecker et al., 2010), which is otherwise difficult to achieve in classic hiPSCs (Zou et al., 2009). One caveat is the mESC-like human cells require persistent reprogramming gene expression, which would interfere with differentiation. However, the authors showed that removal of reprogramming gene expression can revert mESC-like human cells back to a classical hiPSC-like state in the presence of FGF2 and other exogenous factors, albeit at a low frequency (0.01%). With a combination of growth factors (FGF2 and LIF) and a MEK inhibitor, these mESC-like human cells can be converted to a stable state that is indistinguishable from classic hiPSC lines, which do not require sustained expression of the reprogramming transgenes and readily differentiate upon induction (Buecker et al., 2010).

These findings are corroborated by other recent studies that used similar but not identical human cell systems. Ding's group reported that established hESC lines can be converted to mESC-like colonies by LIF and small molecules that promote mESC growth (Xu et al., 2010). Likewise, Jaenisch's group (Hanna et al., 2010) demonstrated generation of mESC-like cells from established iPSCs by using induction of reprogramming transgenes and exogenous factors including LIF. They showed that when hESCs or hiPSCs are converted to a mESC-like state, at least one

reprogramming transgene (KLF4) is required in addition to the standard mESC factors (LIF and small-molecule inhibitors). However, the reprogramming transgenes required for the conversion to the mESC-like state could be replaced by forskolin, a small molecule critical to mouse and human embryonic germ cells (EGC) derivation (reviewed by Kerr et al., 2006). Similar to human EGCs, mESC-like human cells derived from hESCs/hiPSCs in the presence of forsklin (but in the absence of reprogramming transgenes) could only expand for 15–20 passages before they stopped proliferating and differentiated (Hanna et al., 2010).

It is becoming increasingly clear that even the most purified or defined stem populations are heterogeneous: they exist in multiple phenotypically and epigenetically distinct states that are interchangeable (Graf and Stadtfeld, 2008). This view can also apply to in vitro propagated pluripotent stem cells even if they are derived clonally. These recent studies of human and mouse pluripotent cells collectively demonstrate that genetic determinants (which differ across species or various mouse strains), the epigenetic status of a starting cell population, and environmental cues (such as culture conditions) all influence the propensity of pluripotent cells to adopt a stable or metastable state that allows them to self-renew in culture. Those in vitro states may not exist in vivo at all, or may differ between different species even if the reprogrammed cells are derived from comparable origins or developmental stages. Thus, on the basis of these findings, caution should be employed to avoid oversimplification in equating an in vitro state of a particular stem cell line to an in vivo embryonic state, especially when comparing different species. Moreover, the new studies also show that cultured human pluripotent cells can be converted between distinct states that exhibit many of the common attributes of pluripotency but also differ in several defining ways. More practically, the ability to grow hESCs/hiPSCs more robustly, like mESCs, could be useful for many applications, including genetic manipulations. Thus, the realization that human pluripotent stem cells can exist in culture in multiple states will probably help us to utilize them more effectively in future studies and clinical applications.

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The Life of a Cell: Probing the Complex Relationships with the World

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Stem cells live within a complex environment containing an array of signals that impact cell fate. Recently in *Nature Materials*, Huebsch et. al. (2010) probed the role of mechanical properties on stem cell integrin binding and differentiation in three dimensions.

Cells live in a complex world and are exposed to many stimuli in different forms (Figure 1A). What does a cell see and experience in a tissue—the local chemistry, biological signals, texture/morphology, or mechanical environment? It is hard to believe that it has only been 60 years since HeLa cells were first cultured (discussed in Skloot, 2010), opening the door to a plethora of basic and applied research using cells as a tool for discovery and more recently in tissue engineering as building blocks for new tissues. After establishing basic tissue culture techniques, attention has now moved to considering